SPECIFICATION OBJECTION

The specification was objected to because amino acid sequences disclosed in the specification were not referenced by sequence identifiers. Along with this amendment, a substitute Sequence Listing is filed to insert ILRG as SEQ ID NO:2 as disclosed in page 12, line 13, of the specification, and to insert two amino acid sequences, CXDILRG-NH_2 and FMRF-NH_2, as SEQ ID NO: 3 and 4 disclosed in pages 44 and 52 of the specification. Applicants have amended the specification by referring to the amino acid sequence disclosed with SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4.

Applicants submit that any deficiency related to the Sequence Listing Rules has been corrected. Withdrawal of the objection is requested.

Claim Rejections -- 35 U.S.C. 112, First Paragraph

A. Claims 1-12 were rejected as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to use the invention. Applicants respectfully traverse the rejection.

Claims 1-9 are directed toward a gene encoding a pentapeptide, a dormancy-control pentapeptide, a method for preparing the dormancy-control pentapeptide, a biological cell-control agent comprising a pentapeptide as an effective component. Claims 10-12 are directed toward a biological cell-control agent comprising a tetrapeptide as an effective component.

The effectiveness of the pentapeptide, DILRG-NH_2 or DILRG-COOH, in dormancy control and control of cancer cell growth was demonstrated in the specification (e.g. see pages 41 and 47-52). Similarly, the effectiveness of the tetrapeptide, DILRG-NH_2 or DILRG-COOH, in the control of the growth of cancer cells was demonstrated in the specification (e.g. see page 52). As a result, applicants respectfully contend that the Examiner's reasons for the nonenablement rejection will not apply because the biological functions of the pentapeptide or tetrapeptide in dormancy control and cell cycle control has been well described in the specification.

B. Claims 1-12 were rejected because the instant specification does not contain a full written description of the claimed invention. The Office Action asserts that the specification does not describe the structural features, location of introns, exons and open reading frames, and chromosomal location of the gene claimed. Most of the reasons of the rejection apply to only claims drawn to a gene encoding for a protein comprising SEQ ID NO: 1. It appears that the written description rejection should only be applied to claims 1-3, not claims 4-12. Applicants respectfully traverse the rejection because, with the amino acid sequences disclosed (SEQ ID NO:1 or 2), a person skilled in the art would have known the nucleotide sequence of the gene encoding the pentapeptide or tetrapeptide based on the correspondence of amino acids and triplet codons known for *Antheraea yamamai*, so the disclosure in the specification would have provided sufficient written description of the claimed invention.

Withdrawal of the rejections under 35 U.S.C. 112, first paragraph, is requested.

Claim Rejection -- 35 U.S.C. 102

A. Claims 1-5 and 7-9 were rejected under 35 U.S.C. § 102(e) as being anticipated by Todaro et al. (WO 95/13393) and Pearse et al. (WO 95/20661). Applicants respectfully traverse the rejection.

Todaro et al and Pearse et al are both PCT applications, but rejections under 35 U.S.C. § 102(e) must be based on U.S. patents or published U.S. patent applications, not on any PCT applications. Thus, the rejection was based on an improper statutory basis.

Without pointing out the locations of the disclosures of the amino acid sequence of SEQ ID NO:1, the Office Action makes a bare assertion that "Todaro and Pearse both teach a gene/DNA encoding an amino acid comprising SEQ ID NO: 1 (see attached)." Applicants went over the disclosures of Todaro et al and Pearse et al in detail and found out that Todaro et al discloses a hybrid cytokine having a sequence of 192 amino acid residues embracing DILRG (see SEQ ID NO:19, starting at amino acid residue 121) and a gene encoding the hybrid cytokine (see SEQ ID NO:9, starting at nucleic acid residue 361). In relation to human xenotransplantation, Pearse et al discloses a polynucleotide encoding a protein containing DILRG (see Fig. 27, the amino acid sequence starting at residue 98). Since each of Todaro et al and Pearse et al discloses large proteins, there was no specific teaching of the pentapeptide (SEQ ID NO:1) having the C-terminal amino acid amidated or the tetrapeptide (SEQ ID NO:2)

having the C-terminal amino acid amidated as recited in the instant claims. Applicants submit that Todaro et al or Pearse et al does not anticipate the instant claims.

Withdrawal of the anticipatory rejection is requested.

Applicants further contend that Todaro et al or Pearse et al would not have rendered the instant claims obvious because there would have been no desirable reason or motivation to modify the proteins taught by Todaro et al or Pearse et al to arrive at the pentapeptide or tetrapeptide recited in the claims.

B. Claims 1-5 and 7-12 were rejected under 35 U.S.C. § 102(e) as being anticipated by Kubo et al. (WO 94/03205). Applicants respectfully traverse the rejection.

Once again, the Office Action bases the anticipatory rejection on a wrong section of the statute.

According to the Office Action, Kubo et al teaches a peptide capable of binding and inducing an immune response, which may be used in the prevention and treatment of cancer. The peptide comprises an amino acid sequence of SEQ ID NO: 1 (see peptide 5.0044, page 105, table 23(c)) wherein the N-terminal Asp residue has been deleted. However, Kudo et al does not anticipate the instant claims because Kudo et al does not specifically teach the pentapeptide (SEQ ID NO:1 with the C-terminal amino acid amidated) or the tetrapeptide (SEQ ID NO:2 with the C-terminal amino acid amidated).

Withdrawal of the anticipatory rejection is requested.

Applicants further contend that Kudo et al would not have rendered the instant claims obvious because there would have been no desirable reason or motivation to

modify the peptide taught by Kudo et al to arrive at the pentapeptide or tetrapeptide recited in the claims.

Conclusion

In view of the amendment and the above reasoning, Applicants submit that the application is in a condition for allowance. A Notice of Allowance is believed in order.

In the event that the filing of this paper is not deemed timely, Applicants petition for an appropriate extension of time. Any petition fee for the extension of time and any other fees that may be required in relation to this paper can be charged to Deposit Account No. 01-2300, referencing Docket No. 024656-00004.

Respectfully submitted,

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Enclosures: Appendix
Substitute Sequence Listing and Statement
Under 37 CFR 1.821 with paper copy and diskette
Amendment and Fee Transmittal
Petition for Extension of Time

KLW:elp

167705_1.DOC

APPENDIX

Pursuant to 37 CFR 1.121, a marked up version of the amendment is presented.

IN THE SPECIFICATION

Page 10, replace the paragraph starting line 21 with:

The gene Any-RF according to the present invention codes for a protein, which has an amino acid sequence: Asp-Ile-Leu-Arg-Gly [specified as Sequence No. 1 in Sequence Listing] (SEQ ID NO:1), which has a C-terminal amide group and which has a molecular weight of 570.959; possesses dormancy-control activity and is derived from the pre-larvae of *Antheraea yamamai*.

Page 10, replace the paragraph starting line 26 and ending in page 11, line 2, with:

The dormancy-control substance according to the present invention has an amino acid sequence: Asp-Ile-Leu-Arg-Gly [specified as Sequence No. 1 in Sequence Listing] (SEQ ID NO:1), has a C-terminal amide group and has a molecular weight of 570.959.

Page 12, replace the paragraph starting line 4 with:

In addition, the living cell-control agent according to the present invention, for instance, a cancer cell growth-inhibitory agent comprises, as an effective component, a peptide, which has an amino acid sequence: Asp-Ile-Leu-Arg-Gly [specified as Sequence No. 1 in Sequence Listing] SEQ ID NO:1, which has a C-terminal amide group and which has a molecular weight of 570.959. This peptide is one derived from

the pre-larvae of *Antheraea yamamai* as already discussed above and can be prepared by the same method used above.

Page 12, replace the paragraph starting line 11 with:

Furthermore, the living cell-control agent according to the present invention, for instance, a cancer cell growth-inhibitory agent may be one comprising, as an effective component, a peptide having an amino acid sequence: Ile-Leu-Arg-Gly (SEQ ID NO:2) , which corresponds to that specified as [Sequence No. 1 in Sequence Listing] SEQ ID NO:1 from which the N-terminal Asp residue is deleted, which has a C-terminal amide group and which has a molecular weight of 456.58.

Page 15, replace line 16 with:

weight of a synthetic peptide (Asp-Ile-Leu-Arg-Gly-NH_2, SEQ ID NO:1 having the C-terminal amidated) having an amino acid sequence and C-terminal identical

Page 15, replace line 20 with:

weight of a synthetic peptide (Asp-Ile-Leu-Arg-Gly-OH, SEQ ID NO:1) having an amino acid sequence identical to that of the

Page 16, replace the paragraph starting line 1 with:

Fig. 9 is a micrograph showing the morphological change and growth inhibition observed for rat hepatoma cells (dRLh84) when using DILRG-NH_2 (the peptide of SEQ ID NO:1 having the C-terminal amidated) or DILRG-COOH (SEQ ID NO:1).

Page 16, replace the paragraph starting line 4 with:

Fig. 10 is a graph showing the growth-control effect of DILRG-NH_2 (the peptide of SEQ ID NO:1 having the C-terminal amidated) or DILRG-COOH (SEQ ID NO:1) on the rat hepatoma cells (dRLh84) in terms of the relation between the concentration and the viable cell count.

Page 16, replace the paragraph starting line 7 with:

Fig. 11 is a graph showing the growth-control effect of DILRG-NH_2 (the peptide of SEQ ID NO:1 having the C-terminal amidated) or PBS(-) on the rat hepatoma cells (dRLh84) in terms of the relation between the cultivation time and the viable cell count.

Page 16, replace the paragraph starting line 14 with:

Fig. 13 is a graph showing the growth-control effect of DILRG-NH_2 (the peptide of SEQ ID NO:1 having the C-terminal amidated) on the rat hepatoma cells (dRLh84), while comparing it with those observed for other substances.

Page 17, replace the paragraph starting line 6 with:

As has been discussed above, the dormancy-control substance according to the present invention is a novel peptide having a dormancy-control function, 5 amino acid residues thereof from the N-terminal are Asp-Ile-Leu-Arg-Gly (SEQ ID NO:1), and it is a low molecular weight substance (molecular weight: 570.959) and does not have a free oxidized C-terminal, but has a C-terminal carrying an amide group. This is clear from

the fact that only the peptide whose C-terminal carries an amide group possesses such a control function as demonstrated by the biological assay concerning the compounds prepared in Examples as will be described below. This substance can be isolated and purified from, for instance, the pre-larvae of *Antheraea yamamai* or alternatively, it can be synthesized according to the conventional methods since the amino acide sequence thereof is elucidated.

Page 18, replace the paragraph starting line 3 with:

As has been discussed above, the amino acid sequence of the repressive factor of the peptide, which is involved in the maintenance of the dormancy of the pre-larvae of *Antheraea yamamai*, is Asp-Ile-Leu-Arg-Gly-NH_2 (the peptide of SEQ ID NO:1 having the C-terminal amidated). There has not any known peptide of this type of penta-peptide even when the computer research (BLAST and FASTA) is performed and thus the penta-peptide is a novel peptide having dormancy-control activity in the biological world. This is named *Antheraea yamamai*-Repressive Factor (abbreviation: Any-RF). Nobody has ever discovered the peptide comprising 5 amino acid residues and whose C-terminal carries an amide group in the free state in the biological world till the present invention has been completed. However, the amino acid segments identical to the foregoing one: ---Asp-Ile-Leu-Arg-Gly --- can be found in the amino acid sequence of several biological proteins. For instance, the amino acid sequence is identical to that found in the putative 22.1 KD protein (193 amino acid residues) of yeast (i.e. the fragment starting from 166^{th} to 170^{th} amino acid residues) and that found in the precursor (202 amino acid residues) of the human leukemia-inhibitory factor (i.e. the

fragment extending from 142nd to 146th amino acid residues), according to the computer research. However, the functions of the amino acid sequences of these portions have not yet been elucidated at all. In other words, the amino acid sequence in the peptide of the present invention is sandwiched between - and C-terminals, the C-terminal has an amide group and the sequence is thus present in the free state, although the amino acid sequence of the present invention is identical to the fragment present in large protein sequences. Thus, there has never been discovered such an amino acid sequence present in the free state and possessing such a physiological function.

Page 36, replace the paragraph starting line 8 with:

A peptide whose primary structure was completely identical to that of the peptide isolated and purified by the foregoing procedures according to the present invention was prepared by the following method. More specifically, peptides Asp-Ile-Leu-Arg-Gly-NH_2 (the peptide of SEQ ID NO:1 having the C-terminal amidated) (abbreviated as DILRG-NH_2 or RF-NH_2) and Asp-Ile-Leu-Arg-Gly-COOH (SEQ ID NO:1) (abbreviated as DILRG-COOH or RF-COOH) were prepared according to the usual procedures using a peptide synthesizer (PSSM-8 available from Shimadzu Corporation). The purification of these peptides were carried out using a reverse phase column ULTRON VX-ODS (20mm x 250mm, available from Shinwa Kako K.K.) connected to an HPLC system (LC-10A, available from Shimadzu Corporation). The elution was carried out at a flow rate of 8 ml/min and using an acetonitrile concentration gradient (1 to 5% for 0 to 5 minutes; 5 to 60% for 5 to 35 minutes) in the presence of a 0.1% TFA to thus give active fractions. The absorbance at 220 nm was monitored. The purified peptide was mixed

with an equivalent amount of a matrix (50% acetonitril/0.1% TFA saturated with α-CHCA) on the sample plate, followed by drying and confirmation of the purity thereof using MALDI-TOF MS (available from Voyager PerSeptive Biosystems Company).

Page 36, replace the paragraph starting line 24 and ending page 37, line 1 with:

Two kinds of peptides DILRG-NH_2 (the peptide of SEQ ID NO:1 having the C-terminal amidated) (purity: not less than 95%, determined using HPLC and TOF-MS) and DILRG-COOH (SEQ ID NO:1) (purity: not less than 95%, determined using HPLC and TOF-MS) were synthesized according to the foregoing method and they were used in the following Examples.

Page 39, replace lines 16-18 with:

purified product (Fig. 5), a maximum peak at 571.959 for the synthetic peptide: Asp-Ile-Leu-Arg-Gly-NH_2 (the peptide of SEQ ID NO:1 having the C-terminal amidated) (Fig. 6) and a maximum peak at 573.045 for the synthetic peptide: Asp-Ile-Leu-Arg-Gly-COOH (SEQ ID NO:1) (Fig. 7).

Page 42, replace the paragraph starting line 5 with:

Consequently, the amino acid sequence of the repressive factor derived from *Antheraea yamamai* is Asp-Ile-Leu-Arg-Gly-NH_2 (the peptide of SEQ ID NO:1 having the C-terminal amidated) and the molecular weight thereof can be determined by subtracting 1 (mass of a proton) from the measured value of the mass spectrometric peak or 570.959.

Page 44, replace line 20 with:

A peptide: Cys-ε-Acp-Asp-Ile-Leu-Arg-Gly-NH_2 (SEQ ID NO:3) (CXDILRG-NH_2) was

Page 47, replace the paragraph starting line 17 with:

Rat hepatoma cells (dRLh84, 3 x 10^5 cells) were cultured in a culture medium, to which the peptide (DILRG-NH_2 , the peptide of SEQ ID NO:1 having the C-terminal amidated or DILRG-COOH, SEQ ID NO:1) was added in a predetermined amount (0, 50, 100, 150, 200 μg/ml) in the presence of 5% CO_2 at 37°C for 40 hours. Thereafter, the culture medium was treated with trypan blue to determine the viable cell count. The results thus obtained are shown in Fig. 10.

Page 52, replace line 3 with:

supplemented with PBS(-) and FMRF-NH_2 (SEQ ID NO:4), which is widely distributed from

Page 52, replace line 13, with:

the culture medium contained PBS(-) or FMRF-NH_2 (SEQ ID NO:4), the viable cell count ranges from

IN THE CLAIMS

1. (Amended) A gene [Any-RF] coding for a pentapeptide [protein] having an amino acid sequence of [specified as Sequence No. 1 in SEQUENCE LISTING:] Asp-

Ile-Leu-Arg-Gly[, whose C-terminal is amidated] (SEQ ID NO:1) and [having] a molecular weight of 570.959, wherein the C-terminal is amidated.

2. (Amended) The gene [Any-RF] as set forth in claim 1, wherein the pentapeptide has [protein is one having] dormancy-control activity.

3. (Amended) The gene [Any-RF] as set forth in claim 1 or 2, wherein the pentapeptide [protein] is [one] derived from pre-larvae of *Antheraea yamamai*.

4. (Amended) A dormancy-control pentapeptide [substance] having an amino acid sequence of [specified as Sequence No. 1 in SEQUENCE LISTING:] Asp-Ile-Leu-Arg-Gly (SEQ ID NO:1), [whose] a molecular weight of 570.959 and dormancy-control activity, wherein the C-terminal is amidated [, having a molecular weight of 570.959 and having dormancy-control activity].

5. (Amended) The dormancy-control pentapeptide [substance] as set forth in claim 4, wherein the dormancy-control pentapeptide [substance] is [one] derived from pre-larvae of *Antheraea yamamai*.

6. (Amended) A method for preparing a dormancy-control pentapeptide [substance], comprising the steps of

adding an acid-methanol solution consisting of methanol: water: acetic acid to pulverized pre-larvae of an insect;

triturating the resulting mixture;

centrifuging the mixture; and

subjecting the resulting supernatant to reverse phase high performance liquid chromatography and mixing-separation mode high performance liquid chromatography to give a dormancy-control pentapeptide [substance], which has an amino acid sequence of [specified as Sequence No. 1 in SEQUENCE LISTING:] Asp-Ile-Leu-Arg-Gly (SEQ ID NO:1) and a molecular weight of 570.959, [whose] wherein the C-terminal is amidated[, and which has a molecular weight of 570.959].

7. (Amended) A biological cell-control agent comprising, as an effective component, a pentapeptide [peptide] having an amino acid sequence of [specified as Sequence No. 1 in SEQUENCE LISTING:] Asp-Ile-Leu-Arg-Gly (SEQ ID NO:1), [whose] a molecular weight of 570.959, wherein the C-terminal is amidated [and having a molecular weight of 570.959].

8. (Amended) The biological cell-control agent as set forth in claim 7, which [wherein the cell-control agent] is a cancer cell-control agent.

9. (Amended) The biological cell-control agent as set forth in claim 7 or 8, wherein the pentapeptide [peptide] is [one] derived from pre-larvae of *Antheraea yamamai*.

10. (Amended) A biological cell-control agent comprising, as an effective component, a tetrapeptide [peptide] having an amino acid sequence of [corresponds to that specified as Sequence No. 1 in SEQUENCE LISTING from which the N-terminal Asp residue is deleted:] Ile-Leu-Arg-Gly (SEQ ID NO:2)[, whose] and a molecular weight of 456.58, wherein the C-terminal is amidated [and having a molecular weight of 456.58].

11. (Amended) The biological cell-control agent as set forth in claim 10, which [wherein the cell-control agent] is a cancer cell-control agent.

12. (Amended) The biological cell-control agent as set forth in claim 10 or 11, wherein the tetrapeptide [peptide] is [one] derived from pre-larvae of *Antheraea yamamai*.